Rare genetic variants previously associated with congenital forms of long QT syndrome have little or no effect on the QT interval

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Aims
We studied whether variants previously associated with congenital long QT syndrome (cLQTS) have an effect on the QTc interval in a Danish population sample. Furthermore, we assessed whether carriers of variants in cLQTS-associated genes are more prone to experience syncope compared with non-carriers and whether carriers have an increased mortality compared with non-carriers.

Methods and results
All genetic variants previously associated with cLQTS were surveyed using the Human Gene Mutation Database. We screened a Danish population-based sample with available whole-exome sequencing data (n = 870) and genotype array data (n = 6161) for putative cLQTS genetic variants. In total, 33 of 1358 variants previously reported to associate with cLQTS were identified. Of these, 10 variants were found in 8 or more individuals. Electrocardiogram results showed normal mean QTc intervals in carriers compared with non-carriers. Syncope data analysis between variant and non-variant carriers showed that 4 of 227 (1.8%) and 95 of 5861 (1.6%) individuals, respectively, had experienced syncope during follow-up (P = 0.80). There was no significant difference in overall mortality rates between carriers [7/217 (3.2%)] and non-carriers [301/6453 (4.7%)] (P = 0.24).

Conclusion
We present QTc data and register data, indicating that 26 cLQTS-associated variants neither had any effect on the QTc intervals nor on syncope propensity or overall mortality. Based on the frequency of individual gene variants, we suggest that the 10 variants frequently identified, assumed to relate to cLQTS, are less likely to associate with a dominant monogenic form of the disease.

Keywords
Long QT syndrome • LQTS • Exome • False-positive variants • Human Gene Mutation Database

Translational perspective
The present study indicates that the clinicians should be cautious when interpreting a positive genetic result. In cases where a clear cosegregation of phenotype (prolonged QT interval) and genotype exist, the diagnosis of congenital long QT syndrome (cLQTS) is straightforward. In cases where only the proband in the family has a clear phenotype, even in cases where a positive genotype is identified in the LQTS genes, the clinician should be aware of the possibility that the given genetic variant might not necessarily be related to cLQTS in the proband. This should not affect the treatment, but caution is advised in the decision-making of geno-positive relatives without any relevant clinical symptoms.
Introduction

Genetic screening has become an important tool in the identification of patients and family members at risk of having congenital long QT syndrome (cLQTS). It is of great importance that the genetic information of presumed monogenic inherited variants is accurate in order to guide in risk stratification. Counselling based on potentially false-positive variants in the risk stratification process may lead to misdiagnosis, which can have perilous clinical as well as economic and psychological consequences, not only for the patient, but also for the entire family.

Previous studies implicating genetic variants with cLQTS have mainly relied on familial data with or without genotype–phenotype co-segregation and on evidence of electrophysiological effects and/or absence of a given variant in a limited-sized disease-free control population. Although evidence of pathogenicity can be inferred through the methods mentioned earlier, recent studies using whole-exome data have also shown that variants previously thought to be pathogenic in the setting of isolated cardiomyopathy and cLQTS are excessively overrepresented in the NHLBI Exome Sequencing Project population, suggesting that they cannot all be truly causative. Despite that these studies were informative regarding the distribution of rare genetic variants in a large population of 6500 individuals, they were limited by lack of clinical data on the study population as well as biased by the inclusion of different patient cohorts, enriched by selected traits.

In this study, we have analysed randomly selected individuals from the general Danish population, with available phenotypic data. The aim was to investigate (i) whether genetic variants previously associated with cLQTS could be identified in the general population; (ii) whether carriers of genetic variants previously implicated with cLQTS had longer QT intervals compared with non-carriers; (iii) whether carriers of genetic variants previously associated with cLQTS have a higher prevalence of syncope or have increased mortality rates compared with non-carriers; and (iv) the frequency of ‘clinical LQTS’ in carriers and non-carriers, based on the recently published guidelines by Priori et al.

Methods

Identification of cLQTS-associated variants

All genetic variants previously associated with cLQTS were identified. First, we queried to the Human Gene Mutation Database (HGMD) and included variants labelled as ‘Congenital LQTS’ and identified in KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, and KCNJ5. Secondly, a literature search in the PubMed database was performed using the following search terms: “(((((LQ) OR LQTS) OR ‘Long QT Syndrome’[Mesh]) and genetic) OR ‘Genetics’[Mesh]) and mutation) and variant)”, which yielded a total of 599 articles written in English, which were systematically reviewed and included according to the following criteria: (i) family co-segregation; (ii) supporting electrophysiological studies; and (iii) absence in controls. Abstracts and preliminary reports were not included. Familial co-segregation was defined as two or more genotype-positive family members having the same phenotype. Positive functional data were defined as any in vitro or in vivo model, which presented results differing from the wild-type model.

Danish study population

Our study cohort was drawn from the Inter99 study. The Inter99 study is a population study comprising almost 60 000 randomly drawn from an age- and sex-stratified sample of the population. Individuals were included from the age of 30. The Inter99 study was a population-based randomized controlled trial (CT00289237. ClinicalTrials.gov), in which the effects of lifestyle intervention on cardiovascular death were investigated. The Inter99 study has previously been described in detail. Genotyping was performed for approximately 10% of the individuals, and all participants were by self-report of Danish origin. Individuals with available DNA samples underwent exome array genotyping (‘Exome Chip Population’), and a subset of these also had whole-exome sequencing performed (‘Exome Sequencing Population’).

Exome chip study population

From the Inter99 study, 6161 individuals were genotyped using the Illumina Human Exome BeadChip v1.0 genotyping array (‘the exome chip’), which includes approximately 250 000 predominantly rare exonic genetic variants (http://genome.sph.umich.edu/wiki/Exome_Chip_Design). Genotypes were called using GenCall applying a custom-made cluster file based on 6000 samples with high-quality data. Quality control included exclusion of samples showing relatedness (first- and second-degree relatives), extreme inbreeding coefficient (F < 0.1 or F > 0.1), low call rate (<98%), or mismatch between sex status in phenotype and genotype data. All examined variants that were out of Hardy–Weinberg equilibrium were removed (P < 0.05). For variants available on the exome chip, the genotype concordance between the exome sequenced data and the exome chip data is 0.997. The non-concordant variants were removed. European ancestry was confirmed using principal-component analysis (PCA, Supplementary material online, Figure S1).

Whole-exome sequencing population

From the Inter99 study, we identified 870 metabolically healthy individuals on the basis of normal values of fasting plasma glucose levels, body mass index, and blood pressure, who had available electrocardiogram (ECG) measurements and also whole-exome sequencing performed. The exomes were captured using Agilent SureSelect All Exon Kit v2 (46 Mb). Sequencing was performed on Illumina HiSeq 2000. The mean coverage depth across 46 Mb of capture sequence was 56-fold (56 ×), and the average exome coverage per sample was 97.3%. No sample had <90% of its exome covered.

European ancestry was confirmed using PCA. Detailed descriptions of the sequencing procedure as well as quality metrics of the resulting data have been described previously.

ECG data analysis

Upon analysing the ECG, the exome sequenced cohort and the cohort with exome chip data were pooled. All ECGs were digitally recorded and stored in the MUSE Cardiology Information System (GE Healthcare, Wauwatosa, WI, USA) and later processed using version 21 of the Marquette 12SL algorithm. The QT and RR intervals were obtained as a representative median beat from all QRS-T complexes in the 12 leads of the 10 s ECG tracing. QT intervals were adjusted for heart rate using Bazett’s formula (QTc = QT/√RR). Prolonged QTc was defined as QTc ≥ 450 ms for men and ≥ 460 ms for women, according to Schwartz et al. and the 99th percentile of QTc value distribution, and QTc ≥ 470 ms for men and ≥ 480 ms for women, according to a recent article published by Drew et al. In accordance with recently revised definitions of cLQTS, in which a (i) QTc ≥ 480–499 ms and syncope or a (ii) QTc ≥ 500 ms is sufficient for diagnosis of cLQTS, we have...
analysed the rate of such patients among the geno-positive and geno-negative individuals.\textsuperscript{1}

We present sex-adjusted mean QTc intervals for individuals carrying the cLQTS-associated variant and for individuals carrying the wild-type variant (non-carriers) and mean QTc for the overall effect of harbouring cLQTS-associated variants as a group compared with non-carriers. As 5–10\% of the general population may have a QTc interval above the upper limit (QTc \( \geq \) 450 ms in men and \( \geq \) 460 ms in women),\textsuperscript{9} and as means are more sensitive to extreme values, we also compared the burden of extreme values by implicating both the Schwartz guideline criteria and the QTc criteria by Drew et al. mentioned earlier. The latter is particularly helpful to avoid the known overlap in QTc values between LQTS patients and healthy individuals.

### Results

#### Variants identified in Danish exomes and exome chip data

From the literature search, we identified 1358 genetic variants (including missense, nonsense, indels, and splice error mutations) previously associated with cLQTS. Among the 870 patients who had whole-exome sequencing performed, we identified 19 of the 1358 literature-derived genetic variants previously associated with cLQTS. These 19 variants affected 45 heterozygote carriers among the 870 individuals screened, corresponding to a \( \text{'cLQTS genotype prevalence'} \) of 1:19 (45:870) (see Supplementary material online, Table S1).

Among patients with available exome chip data, we identified 23 variants previously associated with cLQTS. These variants were identified in 233 heterozygote carriers and were screened in 6161 individuals, corresponding to a genotype frequency of 1:26. Nine of the 23 variants identified in the population with available exome chip data were also identified in the exome sequenced data. In other words, 14 additional variants were identified exclusively on the exome chip. In total, 33 variants previously associated with cLQTS were identified affecting 243 individuals, and all were missense mutations (see Supplementary material online, Table S1). None of the 243 geno-positive individuals have previously been given the cLQTS diagnosis.

Genetic variation in the 13 cLQTS-associated genes is presented in Supplementary material online, Table S3, with corresponding mean QTc intervals.

#### Effect on QTc interval of cLQTS-associated variants

ECG data with QTc intervals were available for 6088 individuals. Of these, 231 individuals were carriers of 1 cLQTS-associated variant and 5 were carriers of 2 cLQTS-associated variants. The remaining 5861 individuals did not harbour a previously reported cLQTS-associated variant. Age, gender ratio, mean heart rate, and mean QTc interval stratified by carrier status are presented in Table 1.

Of the 33 variants identified in our data, QTc data were available for individuals covering 26 of the variants. All 26 mean QTc intervals were normal according to standard guidelines, when comparing

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of gender distribution, age, heart rate, and QTc intervals in carriers and non-carriers in pooled exome sequencing and exome chip data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier status</td>
<td>N</td>
</tr>
<tr>
<td>Carrier</td>
<td>227</td>
</tr>
<tr>
<td>≥ 8 individuals</td>
<td>198</td>
</tr>
<tr>
<td>&lt; 8 individuals</td>
<td>29</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>5866</td>
</tr>
</tbody>
</table>

N, number of participants; M/F, male/female; HR, heart rate.
mean QTc of cLQTS carriers to non-carriers on a variant-to-variant level (Table 2).

In carriers, 19 of 236 (8.1%) had a QTc above upper limits according to Schwartz et al. and 3 of 236 (1.3%) had a QTc above upper limits according to Drew et al. In non-carriers, 424 of 5861 (7.3%) had prolonged QTc according to the Schwartz criteria and 74 of 5861 (1.3%) according to the Drew criteria, respectively. There was no significant difference in carriers compared with non-carriers regarding the QTc value above respective upper limit (P = 0.64 and 0.99, respectively). Using the recently revised cLQTS criteria presented by Priori et al., none (0/236) in the carrier group and 4/5861 in the non-carrier group met these criteria (P = 0.69). ECG data have also been assessed using Fridric’s formula with similar outcome and presented in Supplementary material online, Table S4.

When observing the overall effect on the mean QTc values when harbouring a cLQTS-associated variant as a group compared with non-carriers, these numbers were almost identical: 425.1 ms in cLQTS carriers and 423.2 ms in non-carriers (P = 0.19). In Figure 1, we present the overall distribution of the QTc intervals in both carriers (blue) and non-carriers (red). The histograms are almost identical with a considerable overlap, and there was no statistical difference (P = 0.19).

Five individuals harbouring two cLQTS-associated variants were identified. These individuals displayed normal QTc intervals (see Supplementary material online, Table S2).

### Variants frequently identified and their effect on the QTc interval

We identified 10 variants present in 8 or more individuals (see Supplementary material online, Table S1). Three of these variants (F2004L in SCN5A, T78M in CAV3, and A257G in SNTA1) were present in 30 or more individuals.

We found 37/6132 individuals who carried the variant A257G in SNTA1. A257G was significantly associated with a longer QTc interval compared with non-carriers (431.3 vs. 423.3 ms; P = 0.04) (Table 2). A tendency for shortening effect on the QTc interval was seen for R1193Q in SCN5A, where we identified 18/6087 carriers

### Table 2 Genetic variants identified in exome sequencing and exome chip data in the Danish study sample presented with mean QTc intervals

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Amino acid</th>
<th>rs#</th>
<th>Number of subjects with available ECGs</th>
<th>Mean QTc interval Danish controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Carrier</td>
<td>Non-carrier</td>
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<tr>
<td>KCNQ1</td>
<td>c.1179G&gt;C</td>
<td>p.K393N</td>
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<td>6076</td>
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<tr>
<td></td>
<td>c.1189C&gt;T</td>
<td>p.R397W</td>
<td>rs19947276</td>
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<td>606</td>
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<tr>
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<td>c.1553G&gt;A</td>
<td>p.R518Q</td>
<td>rs145974930</td>
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<td>SCN5A</td>
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<td>p.A110T</td>
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<td>p.S216L</td>
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<td>p.V1597M</td>
<td>rs19947327</td>
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<tr>
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<td>p.R1626H</td>
<td>rs19947328</td>
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<td>rs19947331</td>
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<td>p.Q1909R</td>
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<td>c.325G&gt;A</td>
<td>p.V1091I</td>
<td>rs77442996</td>
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<tr>
<td>KCNE2</td>
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<td>p.M54T</td>
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<td>CAV3</td>
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<td>p.T78M</td>
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<tr>
<td>SNTA1</td>
<td>c.770G&gt;C</td>
<td>p.A257G</td>
<td>rs56157422</td>
<td>37</td>
<td>6051</td>
</tr>
</tbody>
</table>

*HGMD, Human Gene Mutation Database.

*This individual had a heart rate of 110 b.p.m.
of the variant, however, the difference was not statistically significant (413.6 vs. 423.4 ms; \( P = 0.08 \)).

Registry data on syncope and mortality
Among individuals carrying a previously LQTS-associated variant, 4 out of 227 individuals (1.8%) had a history of syncope. In non-carriers, we identified 95 subjects out of 5861 individuals (1.6%) with a history of syncope, and no significant difference was observed (\( P = 0.80 \)). Of the four individuals carrying an LQTS-associated variant and a history of syncope, two were carriers of the variant A257G in \( \text{SNTA1} \), one carried K393N in \( \text{KCNQ1} \), and one individual carried F2004L in \( \text{SCN5A} \).

In addition, we compared the overall mortality in carriers and non-carriers. In the carrier group, 7/217 (3.2%) individuals died during a median follow-up time of 12.8 years, whereas the corresponding mortality rate in the non-carrier group corresponded to 301/6453 (4.7%, \( P = 0.24 \)).

Discussion
In this study, we identified 33 genetic variants previously associated with cLQTS in a study sample selected to represent the general Danish population. We investigated whether individuals harbouring these cLQTS-associated variants had QTc prolongation compared with non-carriers. Mean QTc intervals for carriers compared with non-carriers were normal, according to the current standard guidelines.\(^9,10\) Carriers of a cLQTS-associated variant did not have an increased propensity to syncope or an increased overall mortality compared with non-carriers.

Variants identified in Danish exomes and exome chip data
The genotype prevalence analyses displayed that 1 in 19 (5.3%) individuals in the whole-exome sequenced study sample (\( n = 870 \)) were carriers of a cLQTS-associated variant and 1 in 26 (3.8%) were carriers in the population with exome chip data (\( n = 6161 \)), respectively. If the prevalence of cLQTS in the general population is set to 1:2000,\(^15\) the identified prevalence in the whole-exome sequenced population and the study sample with exome chip data is almost 80 times higher compared with the phenotype prevalence in the general population. In other words, 3.8–5.3% of the general population are carriers of a potentially pathogenic mutation. This is unexpected, as such a selective overrepresentation of cLQTS-associated variants in a cross-sectional Danish study sample is not in line with the expected prevalence in the general population. Even if taking reduced penetrance of certain variants (for some as low as 25%)\(^16\) into account, an overrepresentation will still be present.

Using the phenotypic criteria presented by Priori et al.,\(^1\) we found that 4/5681 individuals in the non-carrier group had either a QTc of 480–499 ms and a history of syncope or a QTc \( \geq 500 \) ms. None of the geno-positive individuals met these criteria. This corresponds to 4/6088 individuals who have clinical cLQTS in our data, which is in line with the phenotype prevalence presented by Schwartz et al.\(^15\) (1:2000).

QTc interval analysis in variants frequently identified
As many of the identified variants are rare, statistical power for single-variant analysis was limited to variants frequently identified. We have therefore defined an arbitrary cut-off, where variants identified in 8 or more individuals (minor allele frequency \( \geq 0.065% \)) are set as 'variants frequently identified'.

In total, we identified 10 variants frequently identified, and in this study, these variants do not seem to have a pathogenic effect on the QTc interval when inherited in isolation, nor do they dispose to syncope. The average age among cLQTS carriers was 59 (±8) years. In the setting of high-penetrant monogenic causes of cLQTS, the age is therefore past the typical time of disease onset, as cLQTS patients most often develop symptoms at an early age and have experienced major cardiac events by the time of early adulthood.\(^17\) However, according to Goldenberg et al.,\(^18\) the risk of cardiac events remains significant, even after 40 years of age in cLQTS definite cases. Therefore, advanced age in isolation does not exclude a late onset of the disease in a small number of cases.

It can be argued that the abundant presence of these more frequent variants in a general population (devoid of the cLQTS diagnosis), advanced age, harmless QTc intervals, and no difference in syncope susceptibility and mortality indicate that these variants most likely have no or little effect on the QTc interval.

Interpretation regarding the low-frequent variants is less straightforward. These variants can potentially represent pathogenic variants with low or incomplete penetrance, genetic modifiers, or benign bystanders. Of the low-frequent variants, only 1 of 23 is reported with familial co-segregation patterns in the original papers. Certain variants have been associated with cLQTS in a time where the discovery of the variants in LQTS cases and the absence of the same variants in a few hundred controls was enough to label them as pathogenic. In addition, several low-frequent variants have been identified through large-scale screenings of cLQTS patients, in which neither family co-segregation nor functional studies were presented for the individual variants.
Role of functional studies

Information regarding functional studies was obtained through a comprehensive literature search. Functional studies have been conducted on 17/33 variants (51.2%), of which 8/17 (47.1%) were variants frequently identified (variants in 8 or more individuals) in our data. These variants have been investigated extensively and presented with convincing functional data, indicating a probable pathogenic role in cLQTS. However, according to our data derived from healthy individuals, they did not have any apparent effect on the QTc interval, risk of syncope, or all-cause mortality. The most frequently identified variant in our data, denoted T78M in CAV3, is an example of this. T78M is present in 44 individuals who presented with completely normal mean QTc interval in this study. This is interesting as T78M was one of the cornerstone variants in the discovery of LQT9, and its effect has recently been disputed.

A plausible explanation to why cLQTS-associated variants identified in the general population are unable to significantly affect the QTc interval in our study, but can induce an electrophysiological effect in functional studies, could be that functional studies in model systems may not always be representative of in vivo human physiology, and an observed difference in the laboratory may not always have a clinical impact in real life. Therefore, functional studies might oversimplify and overestimate a complex interaction between competing and cooperating factors in the cardiac myocyte, which is extremely difficult to replicate in a laboratory setup. Functional studies should therefore be regarded as a supportive approach in validating a potential pathogenic variant; however, such findings should not stand alone in the validation of whether a genetic variant is pathogenic or not. Focus should be on investigating family pedigrees and clinical history, as well as using large ethnically matched control groups as important additional information.

Importantly, we identified 2.4% (33/1358) of all variants previously associated with cLQTS, which, in other words, means that the vast majority (97.6%) of the cLQTS-associated variants were not identified among normal individuals in this study. We are unable to draw any conclusion for the variants not interrogated in this study regarding susceptibility for developing cLQTS. However, the absence of these (97.6%) variants in our data supports that the variants could indeed represent truly disease-causing variants.

Limitations

As most of the genetic variants identified in our study are rare, we are limited by the sample size, and certainly even larger data sets are needed in order to understand the ‘true’ pathogenicity of some of the abovementioned variants. In addition to exome sequencing data, we used exome chip data, where variants of interest are selected beforehand. Due to this pre-selection of variants, variants not represented on the chip are bypassed.

Patients, who sought medical attention and were hospitalized, were included in the syncope analysis. Individuals who experienced syncope without being admitted to a hospital are therefore bypassed. Using the ICD10 code for syncope, syncope cannot be differentiated from syncope due to arrhythmia or vasovagal episodes as primary cause. In addition, register data regarding syncope were only available from year 1994.

As mentioned earlier, QTc is highly variable in the general population, with up to 15% of supposedly healthy individuals presenting with QTc intervals above reference limits. Furthermore, 25–35% of all genetically confirmed LQTS patients have non-diagnostic QTc intervals. Hence, a cautious approach is important when studying effect of genetic variants on QTc. The pathogenicity of a given genetic variant cannot solely be quantified from ECG data, but must be analysed in concert with clinical data, family history, and data on the prevalence of genetic variants in an appropriate cohort representative for the general population.

It can be argued that the relatively advanced age of 59 (± 8) years among cLQTS carriers and the ‘lack’ of pathogenic QTc intervals are due to ascertainment bias—implicating that carriers of highly pathogenic variants (or combinations of pathogenic variants) die before they reach the age of inclusion (30 years of age). The remaining geno-positive individuals could in theory represent ‘survivors’, with an extraordinary large repolarization reserve, which can potentially rescue a defect in an ion channel by increasing flow through other ion channels.

Conclusion

We identified 33 variants, in 243 heterozygote carriers, in various genes previously associated with cLQTS in a Danish population-based sample with available whole-exome sequencing and exome chip data. Of these, 10 variants were identified in 8 or more individuals. Carriers of previously cLQTS-associated variants found in the present study showed normal mean QTc interval, compared with non-carriers. There was no difference in syncope or overall mortality in carriers compared with non-carriers. Our data indicate that there might be a significant number of low-frequency cLQTS-associated variants, with no or modest effect on the QTc. Caution should be advised with regard to risk stratification and genetic counselling.

Supplementary material

Supplementary material is available at European Heart Journal online.

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